

Materials and Methods: *In vitro*, we examined the effect of AT-101 (kindly provided by Ascenta Therapeutics Inc.) radiation and the combination on apoptosis induction and clonogenic survival in two HNSCC cell lines that expressed the target proteins: UM-SCC-11B (derived from a primary tumor of the larynx) and VU-SCC-OE (derived from a primary tumor of the oral cavity). Apoptosis was determined by bis-benzimide staining to detect morphological nuclear changes and/or by propidium iodide staining and flow-cytometry analysis to quantify sub-diploid apoptotic nuclei. The type of interaction between AT-101 and radiation was evaluated by determining the Combination Index (CI) and isobolographic analysis. In addition, we assessed clonogenic survival upon combined treatment in the VU-SCC-OE cell line. In the clinical study, N07CRH, patients with locally advanced HNSCC, were enrolled in a two-arm trial design with standard radiotherapy/cisplatin treatment combined with concurrent dose-escalating oral AT-101 according to two different schedules, a 2-weeks daily schedule every 3 weeks, and a pulse-dose schedule on 3 consecutive days, every 3 weeks. Blood samples were collected and serum concentrations of AT-101 were determined by HPLC methods.

Results: *In vitro* results showed that AT-101 (10-15 μ M) enhances radiation(5Gy)-induced apoptosis with CI's ranging from 1.1 (additive) to 0.74 (synergistic). Clonogenic survival assays showed a radiosensitizing effect with a DEF₃₇ of 1.3 at concentrations of AT-101 that were markedly lower than used for apoptosis studies. Patients tolerated AT-101 well up to doses of 20 mg. Pharmacokinetic analyses of blood samples taken from the patients at time intervals from 30 minutes up to 24 hours after oral intake showed a dose-dependent increase in serum concentration with peak concentrations up to 300 - 700 ng/ml (0.5 - 1.2 μ M) between 2 and 2.5 hours after intake.

Conclusions: AT-101 is a competent enhancer of radiation-induced apoptosis in HNSCC *in vitro*. In addition, *in vitro* radiosensitization was observed at clinically achievable serum levels. These findings support further evaluation of the combination of AT-101 with radiation in Bcl-2-overexpressing tumors.

PO-1061

Radiosensitisation properties of PI3K/AKT inhibitor GDC-0941 in prostate cancer cells

R. Floyd¹, N. McDermott¹, A. Meunier¹, L. Marignol¹

¹Trinity Centre for Health Sciences, Radiation Therapy Dept., Dublin 8, Ireland Republic of

Purpose/Objective: Radiation therapy is a primary treatment modality for prostate cancer. Over activation of the phosphoinositide 3-kinase (PI3K) pathway causes radioresistance increasing cell survival following radiation, resulting in treatment failure and disease recurrence. Downstream effects of PI3K increase HIF1- α concentrations resulting in high levels of hypoxia in PI3K activated prostate cancer cells. This study investigated the effect of PI3K inhibitor, GDC-0941, on the radiosensitisation of cell lines DU145 and 22Rv1 under hypoxic and normoxic conditions.

Materials and Methods: GDC-0941 was combined with radiation treatment to assess the radiosensitisation effect in DU145 and 22Rv1 cell lines. GDC-0941 was also tested under hypoxic conditions to assess if radiosensitivity was

maintained. Clonogenic assays were used to assess cell survival under the varied treatment conditions.

Results: GDC-0941 was shown to enhance radiosensitivity in both 22Rv1 (2Gy: SER=1.7, p=0.038) and DU145 (2Gy: SER=2, p=0.0025). The radiosensitisation conferred was also significant under hypoxic conditions in 22Rv1 (2Gy: SER=2, p=0.0155) although non-significant in DU145 (2Gy: SER=1.1, p=0.1835). GDC-0941 showed comparable radiosensitisation under both normoxic and hypoxic conditions in both cell lines.

Conclusions: GDC-0941 radiosensitised prostate cancer cells under hypoxic and normoxic conditions.

PO-1062

Radiosensitization of tumor cells by Paclitaxel relies on chromosome missegregation and depends on TPX2

M. Orth¹, C. Belka¹, K. Lauber¹

¹Klinik und Poliklinik für Strahlentherapie und Radioonkologie, Radiation Oncology, München, Germany

Purpose/Objective: The anti-neoplastic compound Paclitaxel (Taxol[®]) is adopted for multiple strategies of cancer treatment encompassing classic chemotherapy on the one side as well as adjuvant treatment settings that combine chemotherapy with other treatment modalities like radiation therapy on the other. The molecular mechanism(s) by which Paclitaxel exerts radiosensitization of tumor cells is not understood in full detail. Moreover, the doses of Paclitaxel that are currently applied in the clinic often coincide with side effects of major severity. Finally, no stratification markers that allow for predicting the responsiveness of tumors towards treatment schedules involving Paclitaxel and radiotherapy are available thus far.

Materials and Methods: Multiple concentrations of Paclitaxel were screened for respective effects on the viability and the proliferation of tumor cells. After identifying low nanomolar doses of Paclitaxel to impact tumor cell proliferation and -viability in a hitherto highly neglected manner, a cohort of tumor cell lines was screened for individual differences in susceptibility towards equivalent doses of Paclitaxel, either administered alone or in combination with irradiation. Based on this screen, a search for new stratification markers was performed.

Results: We show that Paclitaxel at lower nanomolar concentrations effectively sensitizes tumor cells towards ionizing radiation by facilitating high-grade aneuploidization. At such concentrations, Paclitaxel renders the ordinary, bipartite mode of cell division into a highly non-equational, mainly tripartite one thereby facilitating huge levels of aneuploidization and this is frequently followed by a distinct kind of apoptotic cell death. We show that this effect can be correlated with Paclitaxel-dependent radiosensitization of tumor cells since cell lines that are resistant to it are sensitized to lesser extents. We also provide evidence that both, Paclitaxel-dependent aneuploidization and -radiosensitization of tumor cells correlate with the expression levels of AURKA and TPX2, two proteins involved in mitotic spindle assembly, since a knockdown of TPX2 not only rescues the bipartite mode of cell division in the presence of Paclitaxel but also diminishes the radiosensitization effect that is achieved by Paclitaxel.

Conclusions: Here we describe a novel, hitherto largely neglected mode of action exhibited by Paclitaxel. This effect potentially constitutes for both, Paclitaxel's cytostatic - as well as radiosensitizing propensity as revealed by our data and a recent report by Zasadil et al. (2014). Moreover, we identify TPX2, a potential stratification marker not only for combined-modality approaches involving Paclitaxel and radiotherapy but for Paclitaxel-based treatment regimens.

PO-1063

Effect of AZD8931, alone or in combination with radiotherapy, on LoVo cells: comparison with cetuximab and gefitinib

I. Palumbo¹, S. Piattoni², C. Antognelli³, M. Calzuola¹, M. Pirro⁴, F. Bagalia⁴, E. Mannarino⁴, V.N. Talesa⁵, C. Aristei⁶

¹*Radiation Oncology Section, Department of Surgical and Biomedical Sciences University of Perugia, Perugia, Italy*

²*Haematology Section, Department of Medicine University of Perugia, Perugia, Italy*

³*Biosciences and Medical Embryology Section, Department of Experimental Medicine University of Perugia, Perugia, Italy*

⁴*Internal Medicine Angiology and Arteriosclerosis Diseases Section, Department of Medicine University of Perugia, Perugia, Italy*

⁵*Biosciences and Medical Embryology Section, Department of Experimental Medicine University of Perugia, Perugia, Italy*

⁶*Radiation Oncology Section, Department of Surgical and Biomedical Sciences University of Perugia, Perugia, Italy*

Purpose/Objective: Anti-Epidermal Growth Factor Receptor (EGFR) drugs include monoclonal antibodies and tyrosine kinase inhibitors (TKIs). We previously showed that gefitinib (an EGFR TKI) reduced the proliferating capacity of surviving cells after radiotherapy (RT) combined with 5-fluorouracil (Palumbo I et al. Int J Colorectal Dis 2014). The aim of this study was to compare the effect of ADZ8931 (a novel equipotent EGFR, HER2 and HER3 TKI), alone or combined with RT, with cetuximab (an anti EGFR monoclonal antibody) and gefitinib in the LoVo colorectal cancer cell line with a mild EGFR and a low ERBB2 and ERBB3 expression.

Materials and Methods: LoVo cells were treated with 0.8 mM cetuximab, gefitinib and AZD8931 (alone for 5 consecutive days), with fractionated RT (2 Gy daily for 3 consecutive days), or with drugs in combination with RT (starting drugs administration 48 h before RT and continuing it for additional 3 consecutive days, for a total of 5 days). After treatments, cell cycle and apoptosis were evaluated by cytofluorimetric analysis, and the clonogenic capacity by colony forming unit assay (counting and measuring colonies with ImageJ software). Results were evaluated 24h after the end of each treatment.

Results: Our preliminary results showed that all drugs alone slightly increased the apoptotic rate and G1 and G2-M cell cycle phases and, accordingly, reduced the S phase. Interestingly, AZD 8931 induced a more marked reduction in the S phase (the most radioresistant cell cycle phase) compared with cetuximab and gefitinib. Regarding the clonogenic capacity, our data showed that cetuximab reduced colonies number and size by 25% and 50%, respectively, and gefitinib by 35% and 70%, respectively, compared to untreated cells. It is worth of notice that the novel AZD 8931 TKI decreased the colony-forming capacity, in

number and size, by about 10% more than gefitinib. Exposure of cells to RT alone increased the apoptotic rate, enhanced G1 and G2-M cell cycle phases, and reduced colonies number and size by about 70%, compared with untreated cells. RT in combination with all the tested drugs potentiated the above reported observed changes, particularly with AZD8931.

Conclusions: To our knowledge, this is the first study describing the effect of the novel equipotent EGFR, HER2 and HER3 TKI AZD8931 combined with RT. Our data show that AZD8931, most of all the other tested drugs, decreased the growth of LoVo cells. Since we found that AZD8931 markedly reduced the radioresistant S-phase is reasonable to assume that this drug might represent a potentially effective radiosensitizing agent, even in tumor cells expressing mild or low levels of EGFR or ERBB2, ERBB3, respectively.

PO-1064

90y/ 177Lu-radiopeptide therapy and external beam radiation therapy applied to neuroendocrine tumor patients

M. Cremonesi¹, C. Garibaldi¹, M. Ferrari², L. Bodei³, D. Zerini⁴, F. Botta², F. Cattani², B. Jereczek⁴, C. Grana³, G. Pedrolì², R. Orecchia⁵

¹*European Institute of Oncology, Radiation Research, Milano, Italy*

²*European Institute of Oncology, Medical Physics, Milano, Italy*

³*European Institute of Oncology, Nuclear Medicine, Milano, Italy*

⁴*European Institute of Oncology, Radiotherapy, Milano, Italy*

⁵*European Institute of Oncology, Medical Imaging and Radiation Sciences, Milano, Italy*

Purpose/Objective: Peptide receptor radionuclide therapy (PRRT) with ⁹⁰Y-DOTATOC (YD) and ¹⁷⁷Lu-DOTATATE (LuD) has been shown to be effective against neuroendocrine tumors (NETs), with up to 35% response rate and acceptable toxicity profile. In the clinical history of NET patients (pts), external beam radiation therapy (EBRT) may be proposed, as adjuvant in oligometastatic disease or palliative for analgesic purpose. The aim of this study is to assess whether the two radiation therapies are compatible or arise questions about tolerability.

Materials and Methods: Over 807 pts treated with PRRT (1997-2014) for metastatic NETs in our institute, 15% underwent also EBRT. Of these, 17 pts with dosimetry and clinical information for both therapies were selected. They were affected by primary tumor in pancreas (10) and ileum (3) NETs, intestine (1) and lung (3) carcinoids, and had multiple mets in liver (15), bone (9), lymph nodes (7) and lung (3). Fourteen pts received EBRT after PRRT, while 3 before. In 8 cases EBRT was considered adjuvant (see figure) - while palliative for the remaining. Bone mets were irradiated by single post or ant-post fields. In case of oligometastases, stereotactic IMRT or RapidArc treatments were applied, and treatment plans optimized accounting for the dose received by the organs at risk (OARs) during PRRT. The absorbed dose on OARs and tumour (T) were assessed and toxicity explored (CTCAE v4 criteria). For PRRT, kidneys (K) and red marrow (RM) were the OARs; dose to the liver (L) was also evaluated. For EBRT the OARs depended on the T site.